X linked Charcot-Marie-Tooth disease (CMTX1): a study of 15 families with 12 highly informative polymorphisms

S Cochrane, J Bergoffen, N D Fairweather, E Müller, M L Mostacciuolo, A P Monaco, K H Fischbeck, N E Haites

Abstract

X linked dominant Charcot-Marie-Tooth disease (CMTX1) has previously been localised to Xq13-21. Fifteen families were studied using 12 highly informative polymorphisms in the pericentric region of the X chromosome. Phase known recombinations in these families localise the X linked dominant CMT gene to the region distal to DXS106 (Xq11.2-12) and proximal to DXS559 (Xq13.1). These markers flank approximately 2 to 3 Mb of DNA to which GJB1 and CCG1 have already been mapped. A recent report of mutations in the GJB1 gene in subjects with CMTX1 makes this a strong candidate gene.

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Medical Genetics, Department of Molecular and Cell Biology, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD, UK
S Cochrane
N D Fairweather
N E Haites

Department of Genetics, Kaiser Permanente Medical Group, San Jose, CA, USA
J Bergoffen

Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA
J Bergoffen
K H Fischbeck

ICRF Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK
N D Fairweather
A P Monaco

Genetics Laboratory, Department of Biology, University of Padova, Padova, Italy
E Müller
M L Mostacciuolo

Correspondence to Dr Haites.

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X linked Charcot-Marie-Tooth disease (CMTX) is a clinically heterogeneous group of diseases with both dominant and recessive inheritance. The dominant form (CMTX1) was provisionally assigned to the proximal long arm of the X chromosome in 1985.¹ This has been confirmed by the work of several other groups in many families,²⁻⁸ and the gene has subsequently been localised to the segment of proximal Xq between PGKP1 (Xq11.2-12) and DXS72 (Xq21.1).⁹ In addition, other workers have described recessive forms, one associated with mental retardation, provisionally mapping to Xp22.2 and the other to Xq26.¹⁰

CMTX1 was once considered rare in comparison to autosomal dominant forms. However the ability to test for the duplication at chromosome 17p11.2-12,^{11,12} characteristic of the majority of HMSN1A families, has allowed diagnosis to be reconsidered in many smaller families, whose inheritance and clinical features were not inconsistent with either mode of inheritance.

We report here a study performed on 15 X linked dominant families (figs 1A, 1B, and 2) using 12 recently described highly informative polymorphisms (fig 3) spanning the region of interest on the X chromosome.

Materials and methods

The families were identified at regional neurological and genetic clinics in the United Kingdom, the USA, and Italy. Typically, affected

males showed pes cavus, distal muscle wasting, and weakness which is progressive from the mid teens. Diminished or absent tendon reflexes are also present, initially in the lower limbs but eventually progressing to the upper limbs, with variable sensory loss. In addition, affected males within these families had median nerve conduction velocities of less than 38 m/sec, a feature also observed in dominantly inherited CMT type 1. Wide variation in the severity of clinical signs was found in carrier females who had later onset of symptoms, with some obligate carriers being asymptomatic.

Genomic DNA was isolated from peripheral white blood cells using a modified standard technique.¹³ Southern blot analysis was performed using the markers M27 β (DXS225)¹⁴ and PGK1.¹⁵ PCR amplification of microsatellites at HAR,¹⁶ PGKP1,¹⁷ DXS106,¹⁸ DXS135,¹⁹ DXS453,²⁰ DXS559,²¹ DXS227,¹⁸ DXS56 and PGK1,²² DXS441,²³ and DXYS1²⁴ was performed according to the conditions previously described.

Affected members of all families were also probed with pVAW409R3a,¹¹ to confirm the absence of the chromosome 17p11.2-12 duplication.

Results and discussion

All the families show a pattern of inheritance consistent with an X linked disease (that is, no male to male transmission). DNA analysis of affected members showed no indication of the duplication at 17p11.2-12, increasing the likelihood of X linked inheritance.

Not all of the polymorphic markers studied were informative in every family. As expected, there were fewer recombinants detected in the smaller pedigrees. Clinically normal females who were not obligate carriers were excluded from the analysis. The individual recombination events which did occur in specific families are shown in fig 4. These recombinants localise the X linked CMT gene to the region distal to DXS106 (Xq11.2-12)^{19,26} and proximal to DXS559 (Xq13.1).²⁵ These markers flank approximately 2 to 3 Mb of DNA (A P Monaco, personal communication, 1993).

For diagnostic purposes, the locus DXS453 would therefore appear to be a useful marker for the disease, with no recombinations at this site having yet been detected.

Two genes have already been mapped to the region Xq13.1, GJB1²⁷ and CCG1.^{26,28} The CCG1 (cell cycle G1 phase defect) gene com-

Figure 1 (A) Pedigree of Scottish family 8206. (B) Pedigrees of eight British and one Italian families.

Figure 2 Pedigrees of five American families.

Figure 3 Schematic map of the X chromosomal region Xp21.1-Xq21.3, showing loci segregated into 15 different intervals,¹¹ with relevant marker positions.

Figure 4 Recombination analysis using variable number simple sequence repeats (VSSR) and restriction fragment length polymorphisms (RFLP). The markers used are listed in chromosomal order²⁵ on the left. Across the top, the numbers represent carrier females with informative meioses. The vertical lines represent X chromosomes that have undergone phase known recombination during meiosis. Open circles represent non-recombinant loci, and closed circles represent recombinations that have occurred.⁹ A horizontal bar denotes an uninformative result. The arrows point in the direction in which the X linked HMSN gene must therefore lie.

plements a hamster cell cycle mutation and is therefore required in normal cell growth and division. Its functional presence allows cells to pass out of the G1 phase of cell cycle events, which in turn commits them to completing the S, G2, and M phases.²⁹ GJB1 (gap junction protein β 1), otherwise known as Connexin32 (Cx32), is, as its name suggests, a gap junction protein of molecular weight 32 kDa. Cx32 is widely expressed in human tissues³⁰ and mutations within this gene have been found in subjects with CMTX1 making this a strong candidate gene for this disease.³¹

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